

To beam or not to beam?

Answers to potential radiotoxicity issues in longitudinal mouse lung μ CT studies

Greetje Vande Velde¹, Jennifer Poelmans¹, Ellen De Langhe², Ria Bogaerts³, Rik Lories², Uwe Himmelreich¹

¹ Biomedical MRI unit/ MoSAIC, KU Leuven, Herestraat 49 O&N1 box 505, B-3000 Leuven, Belgium; ² Laboratory of Tissue Homeostasis and Disease, KU Leuven;

³ Laboratory of Experimental Radiotherapy, KU Leuven

Aims

Human lung diseases are among the most important causes of morbidity and mortality in the world. Chronic obstructive pulmonary disease (COPD), lung fibrosis and emphysema - either without a known cause or secondary to other diseases such as infections or autoimmune disorders – are examples of devastating and life threatening conditions for which effective treatments are still lacking. Even when sometimes the cause is obvious (e.g. cigarette smoking...), many aspects of the pathophysiology of lung diseases remain to be understood in order to find targets for effective treatment. To unravel disease processes in the lung and to develop new therapeutic strategies in a preclinical setting, animal models (e.g. rat, mouse, ferret...) are indispensable and widely used [1]. In these animals, the resulting lung pathology is routinely quantified by labor-intensive end-stage histological assessments that require many animals. Although histological techniques will remain essential to unravel pathogenesis at a molecular and cellular level, they lack the ability to provide dynamic information on disease progression and potential therapeutic effects. As the course of disease progression and potential response to therapy in rodent models shows substantial inter-individual variation, non-invasive techniques are indispensable to dynamically monitor disease development and progression to establish the kinetics of pathogenic events or treatment effects for each animal individually. At present, imaging tools for the evaluation of lung disease with good temporal and spatial resolution *in vivo* are limited. μ CT has proven to be an efficient and safe non-invasive method to provide dynamic information on lung fibrosis and emphysema progression in mice [2]. However, to extend μ CT scanning protocols to animal models that require more frequent scanning or over longer periods of time (e.g. in transgenic mouse models with slow and/or unpredictable disease onset), radiotoxicity issues that could interfere with the μ CT read-out for the lung must be considered. As radiation-induced lung fibrosis is an important manifestation of radiotoxic damage, this is a particularly relevant potential confounding factor when using μ CT in pulmonary research. Moreover, when imaging the lungs, long scanning times are necessary to include respiratory gating in order to avoid motion artifacts due to breathing. Therefore, x-ray doses and hence the risks for radiation-induced lung injury tend to be higher in lung CT studies. As studies assessing radiotoxicity issues when using μ CT for longitudinal mouse lung imaging are currently lacking, we aimed at evaluating the potential radiotoxicity of repeated retrospectively gated lung μ CT scans for different long-term imaging protocols and validated our results with histochemical techniques to evaluate possible radiotoxicity-induced lung fibrosis.

Method

We used 48 adult male C57Bl/6 mice of 8 weeks old, kept on food and water *ad libitum*. The mice were divided in 4 experimental (E1 - E4) and 4 control groups (C1 - C4) (n = 4 per group) and subjected to different CT imaging schedules as follows:

- Group E1: μ CT 1 x per week during 5 weeks
- Group E2: μ CT 2 x per week during 5 weeks
- Group E3: μ CT 1 x per week during 12 weeks
- Group E4: μ CT 2 x per week during 5 weeks, incl. 1 x high-dose scan in week 3.
- Group C1: non-scanned controls, 1x CT at 5 weeks
- Group C2: non-scanned controls, 1x CT at 12 weeks
- Group C3: age-matched never scanned controls at 5 weeks
- Group C4: age-matched never scanned controls at 12 weeks.

Retrospectively gated μ CT images were acquired on a dedicated small animal μ CT scanner (SkyScan 1076, Bruker micro-CT) with the following parameters: 50 kV, 0.5 mm Al filter, 200 μ A source current, 35 μ m isotropic resolution, 120 ms exposure time, 9 projection images per 0.7° rotation step and FOV covering the lung. For the high-dose scan, exposure time - and hence x-ray dose - was tripled to 450 ms. Image analysis, segmentation of CT data and quantification of aerated lung volume were performed with custom written algorithms using SkyScan software as described before [2] with minor modifications. After the last imaging time point, mice were sacrificed, *ex vivo* CT data were acquired and the lungs were isolated for histological analysis and quantification, *i.e.* scoring of the extent of lung fibrosis by the method of Ashcroft on HE-stained lung sections and quantification of the collagen content of the lung by hydroxyproline assay as described before [2].

Radiation dose per scan was measured in two independent ways by introducing an ionization chamber in the scanner and by placing thermoluminescent dosimeters (TLDs, n = 3) in the scanner while using the above mentioned scanning protocol. Lung dose was measured *ex vivo* by introducing TLDs (n = 3) in the lung of a sacrificed mouse, skin dose by placing TLDs (n = 3) on the skin of a mouse. The measurements were repeated three times and reported as mean \pm SD.

Results

The average total dose for one retrospectively gated μ CT scan as measured by the ionization chamber was found to be 1.64 ± 0.04 Gy. This value was used to calibrate the TLD measurements, resulting in a measured skin dose of 1104 ± 240 mGy and a measured lung dose of 813 ± 103 mGy (**Figure 1**).

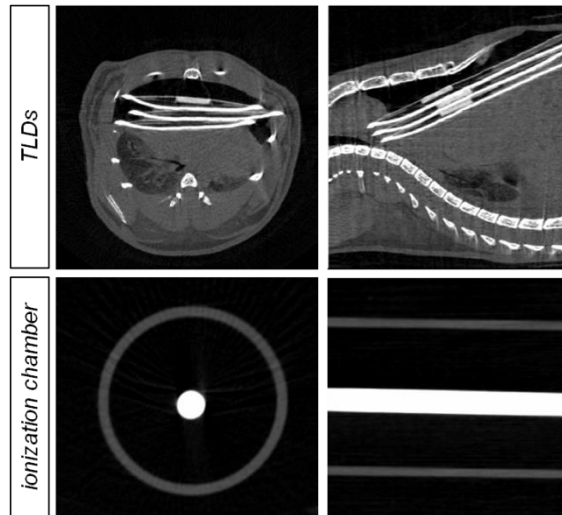


Figure 1: dosimetry. Axial and sagittal CT images of a mouse with TLDs at the level of the lungs (top row) and of the ionization chamber (bottom row) for measuring radiation dose of a scan.

For all experimental groups (E1 – E4), no differences in mean aerated lung volume segmented from the CT data compared to the corresponding control mice groups (C1 – C2) could be found. Upon visual inspection of the *in vivo* CT data for individual mice, no focal spots of increased density that may correspond to fibrotic areas could be identified as compared to controls. No overt signs of possible other lung injuries could be appreciated from the individual *in vivo* CT images (**Figure 2**). Also from *ex vivo* CT images, no signs of lung injury could be appreciated. Taken together, the data indicate that no radiation-induced lung fibrosis could be detected by μ CT in the current setup.

To corroborate the μ CT data, lung sections will be carefully analyzed and the collagen content of lung samples quantified to exclude possible fibrotic lung injuries that might not have reached the detection threshold of the μ CT.

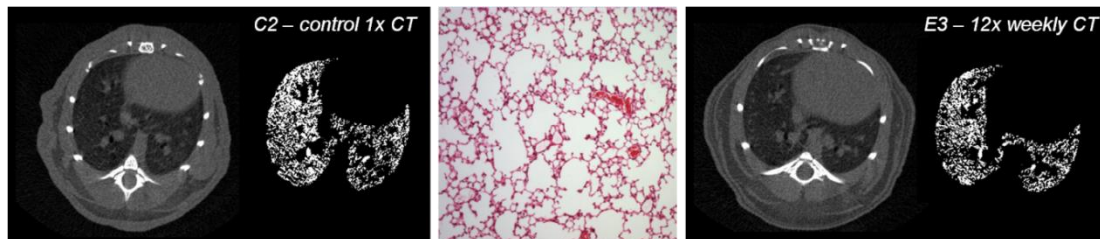


Figure 2: CT images and histology. Axial lung μ CT images acquired at 12 weeks after baseline and corresponding binary images segmented for aerated lung content of a control (left panels) and weekly-scanned mouse (right panels). In the middle panel, an HE-stained histological section showing undamaged lung tissue.

Conclusion

Despite the relatively large radiation dose that we measured during acquisition of a lung μ CT scan, mice do not show signs of radiation-induced lung fibrosis that would potentially interfere with CT read-out for the lungs when scanned weekly during 5 and up to 12 weeks. Doubling the scanning frequency during 5 weeks remained without symptoms, indicating that the time resolution of CT scanning can be safely increased within the given time frame. A single tripling of the radiation dose also

stayed within the limits of the mouse's repair capacity and stayed without detectable symptoms after 5 weeks of scanning. This result suggests that, for instance, a failed scan can be safely repeated without jeopardizing the experimental outcome.

To the best of our knowledge, this is the first study assessing potential radiotoxic damage during longitudinal μ CT scanning, showing that longitudinal monitoring of mouse lungs using μ CT is safe using the here evaluated imaging protocols. This opens perspectives for the long-term monitoring of lung conditions such as fibrosis, emphysema... and therapeutic response on an individual basis with high spatial and temporal resolution, without any concerns for radiation toxicity.

References:

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2. Ellen De Langhe, Greetje Vande Velde, Jeroen Hostens, Uwe Himmelreich, Benoit Nemery, Frank Luyten, Jeroen Vanoirbeek, Rik Lories (2012). Quantification of Lung Fibrosis and Emphysema in Mice Using Automated Micro-Computed Tomography. PLoS ONE 7 (8):e43123.